

CLAIMS

What is claimed is:

1 1. An apparatus for non-electrophoretic determination of the presence of at  
2 least one analyte in each of n flowable samples, said apparatus comprising:  
3 a housing having a cavity formed therein;  
4 n filtrate-receiving vessels positioned within the cavity of said housing;  
5 n membrane components, each of said membrane components being  
6 positioned in association with one of said filtrate-receiving vessels;  
7 n sample-receiving wells, each of said sample-receiving wells being  
8 positioned in association with one of said membrane components such that sample  
9 placed within a particular sample receiving well may be caused to filter through the  
10 associated membrane component, and a filtrate which emerges from that  
11 membrane component will be received within the associated filtrate-receiving  
12 vessel;  
13 a lid for sealing each of said sample receiving vessels and said cavity of said  
14 housing;  
15 a differential pressure source to cause a pressure differential between each  
16 of said sample-receiving wells and each of said filtrate-receiving vessels, said  
17 pressure differential being operative to drive each sample through the associated  
18 membrane component and the resultant filtrate into the associated filtrate-receiving  
19 vessel.

1 2. The apparatus of Claim 1 wherein said pressure source provides  
2 negative pressure within the cavity of said housing so as to pull the filtrate through  
3 each membrane component.

1 3. The apparatus of Claim 1 wherein said pressure source provides  
2 positive pressure within the sample wells so as to push the filtrate through each  
3 membrane component.

Sub 034  
1 4. The apparatus of Claim 2 further comprising:  
2 n air-inlet openings formed in said apparatus, one of said air inlet openings  
3 being associated with each one of said sample-receiving wells, such that when a  
4 particular sample-receiving well becomes empty air will be drawn through the  
5 associated air inlet opening.

1 5. The apparatus of Claim 1 wherein the differential pressure source  
2 comprises a pump which is integral of the test apparatus.

1 6. The apparatus of Claim 5 wherein said pump integral of the apparatus  
2 is a vacuum pump which is incorporated within said housing.

Sub 034  
1 7. The apparatus of Claim 1 wherein at least some of said membrane  
2 components have portions formed of a first hard material, and portions formed of  
3 a second elastomeric material, the portions formed of said elastomeric material  
4 being at locations which abut against neighboring components of the apparatus to  
5 provide substantially air tight sealing therebetween.

1 8. The apparatus of Claim 7 wherein said first and second materials are  
2 co-molded by shooting both said first and second materials into a single mold.

1 9. The apparatus of Claim 1 wherein said membrane modules are plate-  
2 type membrane modules having a plurality of discrete sample flow openings formed  
3 therein with membranes being disposed transversely within each such sample flow  
4 opening.

1 10. The apparatus of Claim 1 wherein said membrane modules are  
2 individual membrane modules, each having a single sample flow opening formed  
3 therein with a membrane positioned transversely within said sample flow opening.

1 11. The apparatus of Claim 9 wherein at least some of the plate-type  
2 membrane modules are provided with engagement members whereby they may be  
3 selectively engaged to and disengaged from a neighboring membrane module of  
4 other adjacent component of the apparatus.

1 12. The apparatus of Claim 11 wherein said engagement members  
2 comprise latches and corresponding latch engagement notches.

1 13. The apparatus of Claim 10 wherein at least some of the individual  
2 membrane modules are provided with engagement members whereby they may be  
3 selectively engaged to and disengaged from a neighboring membrane module of  
4 other adjacent component of the apparatus.

1 14. The apparatus of Claim 13 wherein said engagement members  
2 comprise projections and corresponding projection-receiving slots for bayonet-type  
3 connection.

1 15. The apparatus of Claim 13 wherein said engagement members  
2 comprise helical threads for screw-type connection.

1 16. The apparatus of Claim 9 where in at least some of the plate-type  
2 membrane modules have orientation restricting registry surfaces formed thereon to  
3 deter stacking of the membrane modules in incorrect orientation.

1 17. The apparatus of Claim 9 where in at least some of the plate-type  
2 membrane modules have handles formed thereon to facilitate grasping and  
3 separation of the membrane modules.

1 18. A system for non-electrophoretic determination of at least a first  
2 analyte contained within a matrix, said system comprising:

3 a first membrane module having a membrane which is operative to  
4 prevent some of the matter of said matrix from passing therethrough, while  
5 allowing a filtrate containing said first analyte to pass therethrough;

6 a first vessel positioned in relation to said first membrane so as to  
7 receive said filtrate therein; and,

8 at least one reagent which is combinable with said filtrate in said  
9 receiving vessel to provide a reagent-filtrate admixture containing said first  
10 analyte and from which said first analyte may be determined.

1 19. The system of Claim 18 for detection of first and second analytes  
2 present within said matrix, said system further comprising:

3 a second membrane module interposed between said first membrane  
4 module and said first receiving well, said second membrane having a  
5 membrane which will capture and hold said second analyte while allowing a  
6 sub-filtrate containing said first analyte to pass therethrough and into said  
7 first receiving well;

8 a second receiving vessel which is positioned in relation to said  
9 second membrane after said second analyte has been captured on said  
10 second membrane, such that said second analyte may be eluted from said  
11 second membrane to provide an eluant which contains said second analyte,  
12 within said second vessel;

13 at least one second reagent which is combinable with the eluant in  
14 said second vessel to provide a reagent-eluant admixture from which said  
15 second analyte may be determined.

1 20. The system of Claim 19 further for determination of first, second and  
2 third analytes present within said matrix, said system further comprising:

3 a third membrane module initially interposed between said second  
4 membrane module and said first vessel, said third membrane module having  
5 a third membrane which will capture said third analyte from the sub-filtrate

6 which has passed through said second membrane such that a sub-filtrate  
7 containing said first analyte will be received in said first receiving vessel;

8 a third receiving vessel which is positioned in relation to said third  
9 membrane after said third analyte has been captured on said third  
10 membrane, such that said third analyte may be eluted from said third  
11 membrane to provide an eluant which contains said third analyte, within said  
12 third vessel;

13 at least one second reagent which is combinable with the eluant in  
14 said third vessel to provide a reagent-eluant admixture from which said third  
15 analyte may be determined.

1 21. The system of Claim 22 for determination of n analytes contained in  
2 said matrix, said system further comprising:

3 n membranes interposed in series between said third membrane and  
4 said first receiving well, each of said n membranes being operative to  
5 capture and hold one of said n additional analytes while allowing a sub-sub-  
6 filtrate containing said first analyte to pass into said first receiving well;

7 n receiving vessels which are separately positioned in relation to each  
8 of said n membranes after said n analytes have been captured on said n  
9 membranes, such that said n analytes may be eluted from said n  
10 membranes to provide, within each of said n vessels, an eluant which  
11 contains at least one of said n analytes,;

12 at least one reagent which is combinable with the eluant in each of  
13 said n vessels to provide n reagent-eluant admixtures from which each of  
14 said n analytes may be determined.

1 22. The system Claim 18 for use in determining at least one sub-  
2 detectable analyte which is present in said matrix at a concentration which is less  
3 than the desired concentration for the intended determination of said analyte, said  
4 system further comprising:

an analyte-concentrating membrane module having a membrane which will capture said sub-detectable analyte while allowing a sub-filtrate which is substantially free of said sub-detectable analyte to pass into said vessel;

a sub-detectable analyte receiving vessel which is positioned in relation to said analyte-concentrating membrane after said sub-detectable analyte has been captured on said analyte concentrating membrane, such that said sub-detectable analyte may be eluted from said analyte concentrating membrane to provide an eluant which contains said sub-detectable analyte at a concentration which is suitable for detection, within said sub-detectable analyte receiving vessel;

at least one reagent which is combinable with the eluant in said sub-detectable analyte receiving vessel to permit determination of the sub-detectable analyte in the eluant-sub-detectable analyte admixture.

23. The system of Claim 18 wherein said first analyte is free fatty acid, and wherein:

said first membrane comprises a microporous membrane which will prevent a portion of said matrix from passing therethrough, while allowing free fatty acids to pass therethrough in said filtrate; and,

said reagent comprises xylenol orange, thereby providing a xylenol orange-filtrate admixture in said first vessel, free fatty acid being determinable within said xylenol orange-filtrate admixture.

24. The system of Claim 18 wherein said first analyte is free fatty acid and wherein the sample is subjected to stress prior to free fatty acid determination, and wherein:

said system further comprises a stress reagent which is combinable with a sample of the matrix to promote the formation of free fatty acids therein;

16 said first membrane comprises a microporous membrane which will  
17 prevent a portion of said stressed matrix from passing therethrough, while  
18 allowing the free fatty acids to pass therethrough in said filtrate; and,

19 said reagent comprises xlenol orange, thereby providing a xlenol  
20 orange-filtrate admixture in said first vessel, free fatty acid being  
21 determinable within said xlenol orange-filtrate admixture.

1 25. The system of Claim 18 wherein said first analyte is lipid peroxide and  
2 wherein the sample is subjected to stress prior to lipid peroxide determination, and  
3 wherein:

4 said system further comprises a stress reagent which is combinable  
5 with a sample of the matrix to promote the formation of lipid peroxides  
6 therein;

7 said first membrane comprises a microporous membrane which will  
8 prevent a portion of said stressed matrix from passing therethrough, while  
9 allowing the free fatty acids to pass therethrough in said filtrate; and,

10 said reagent is selected from the group of reagents consisting of:

11 xlenol orange with acidified iron: and,

12 reduced hemoglobin;

13 said second reagent being combinable with the filtrate in the first vessel to  
14 provide a reagent-filtrate admixture from which lipid peroxides may be  
15 determined.

1 26. The system of Claim 18 wherein said first analyte is polyphenol, and  
2 wherein:

3 said first membrane comprises a microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 free fatty acids to pass therethrough in said filtrate; and,

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reduced hemoglobin;

said second reagent being combinable with the eluant in the second vessel to provide an eluant-reagent admixture from which lipid peroxides may be determined.

29. The system of Claim 19 wherein said first analyte is polyphenol and said second analyte is free fatty acid, and wherein:

said first membrane comprises microporous membrane which will prevent a portion of said matrix from passing therethrough, while allowing polyphenols and free fatty acids to pass therethrough;

said second membrane is a membrane which captures polyphenols while allowing free fatty acids to pass therethrough;

said first reagent comprises xylenol orange, which when mixed with the filtrate in the first vessel will provide for determination of free fatty acids; and,

said second reagent comprises folin ciocalteau, which when mixed with the eluant in the second vessel will provide for determination of polyphenols therein; and,

30. The system of Claim 19 wherein said first analyte is polyphenol and said second analyte is lipid peroxides, and wherein:

said first membrane comprises microporous membrane which will prevent a portion of said matrix from passing therethrough, while allowing lipid peroxides and free fatty acids to pass therethrough;

said second membrane is a membrane which captures lipid peroxides while allowing free fatty acids to pass therethrough;

said first reagent comprises folin ciocalteau, which when mixed with the filtrate in the first vessel will provide for determination of polyphenols therein; and,

12 said second reagent is said reagent is selected from the group of  
13 reagents consisting of:

14 xlenol orange with acidified iron: and,  
15 reduced hemoglobin;

16 said second reagent being combinable with the eluant in the second vessel  
17 to provide an eluant-reagent admixture from which lipid peroxides may be  
18 determined.

1 31. The system of Claim 19 wherein said first analyte is all compounds  
2 having an unsaturated c=c bond and said second analyte is lipid peroxides, and  
3 wherein:

4 said first membrane comprises microporous membrane which will  
5 prevent a portion of said matrix from passing therethrough, while allowing  
6 compounds having c=c bonds and lipid peroxides to pass therethrough;

7 said second membrane is a membrane which captures lipid peroxides  
8 while allowing other compounds having c=c bonds to pass therethrough;

9 said first reagent comprises iodide which when mixed with the filtrate  
10 in the first vessel will provide for determination of compounds having c=c  
11 bonds, and

12 said second reagent is said reagent is selected from the group of  
13 reagents consisting of:

14 xlenol orange with acidified iron: and,  
15 reduced hemoglobin,

16 said second reagent being combinable with the eluant in the second vessel  
17 to provide an eluant-reagent admixture from which lipid peroxides may be  
18 determined.

1 32. The system of Claim 19 wherein said first analyte is all compounds  
2 having an unsaturated c=c bonds and said second analyte is malonaldehydes, and  
3 wherein:

4           said first membrane comprises microporous membrane which will  
5 prevent a portion of said matrix from passing therethrough, while allowing  
6 compounds having  $c=c$  bonds and malonaldehydes to pass  
7 therethrough;

8           said second membrane is a membrane which captures lipid  
9 peroxides while allowing other compounds having  $c=c$  bonds to pass  
10 therethrough;

11           said first reagent comprises iodide, which when mixed with the  
12 filtrate in the first vessel will provide for determination of compounds  
13 having  $c=c$  bonds; and,

14           said second reagent is methyl indole which when combined with the  
15 eluant in the second vessel will provide an eluant-reagent admixture from which  
16 malonaldehydes may be determined.

1           33. The system of Claim 19 wherein said first analyte is lipid peroxide  
2 and said second analyte is histamine, and wherein:

3           said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 polyphenols and lipid peroxides to pass therethrough;

6           said second membrane is a membrane which captures histamine  
7 while allowing lipid peroxides to pass therethrough;

8           said first reagent is said reagent is selected from the group of  
9 reagents consisting of:

10                   xlenol orange with acidified iron; and,

11                   reduced hemoglobin;

12           to provide a filtrate-reagent admixture from which lipid peroxides may be  
13 determined; and,

14           said second reagent comprises diamine oxidase and xlenol  
15 orange with acidified iron, for determination of histamine in said eluant-  
16 reagent admixture.

1        34.    The system of Claim 19 wherein said first analyte is malondialdehydes and  
2        said second analyte is sulfite, and wherein:

3                said first membrane comprises microporous membrane which will  
4                prevent a portion of said matrix from passing therethrough, while allowing  
5                malonaldehydes and sulfites to pass therethrough;

6                said system further comprises an intermediate membrane positioned  
7                between said first membrane and said second membrane, said intermediate  
8                membrane being a membrane which will capture pigments and metals, while  
9                allowing malonaldehydes and sulfites to pass therethrough;

10               said second membrane is a membrane which captures  
11               malondialdehydes while allowing sulfites to pass therethrough;

12               said first reagent is xlenol orange with acidified iron to provide a  
13               reagent filtrate admixture from which sulfites may be determined; and,

14               said second reagent comprises methyl indole to provide a reagent  
15               eluant admixture from which malondialdehyde may be determined.

1        35.    The system of Claim 19 wherein said first analyte is histadine and said  
2        second analyte is histamine, and wherein:

3                said first membrane comprises microporous membrane which will  
4                prevent a portion of said matrix from passing therethrough, while allowing  
5                malonaldehydes and sulfites to pass therethrough;

6                said second membrane is a membrane which captures histamine  
7                while allowing histadine to pass therethrough;

8                said first reagent is tetrabromophenol blue to provide a reagent-filtrate  
9                admixture from which histadine may be determined; and,

10               said second reagent comprises diamine oxidase and xlenol orange  
11               with acidified iron, for determination of histamine in said eluant-reagent  
12               admixture.

1           36. The system of Claim 19 wherein said first analyte is all amines  
2 other than histamine and said second analyte is histamine, and wherein:

3           said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 amines including histamine to pass therethrough;

6           said second membrane is a membrane which captures amines other  
7 than histamine while allowing histamine to pass therethrough;

8           said first reagent is diamine oxidase and xylanol orange with acidified  
9 iron to provide a reagent-filtrate admixture from which histamine may be  
10 determined; and,

11           said second reagent comprises xyldinyl blue, for determination of  
12 amines other than histamine in said eluant-reagent admixture.

1           37. The system of Claim 19 wherein said first analyte is aldehydes and  
2 said second analyte is bisulfites, and wherein:

3           said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 amines including histamine to pass therethrough;

6           said second membrane is a membrane which captures aldehydes  
7 while allowing bisulfite to pass therethrough;

8           said first reagent is xylanol orange with acidified iron to provide a  
9 reagent-filtrate admixture from which sulfites may be determined; and,

10           said second reagent comprises methyl indole, for determination of  
11 malonaldehydes in said eluant-reagent admixture.

1           38. The system of Claim 19 wherein said first analyte is protein and said  
2 second analyte is aldehyde, and wherein:

3           said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 proteins and aldehydes to pass therethrough;

said second membrane is a membrane which captures aldehydes while allowing proteins to pass therethrough;

said first reagent is Commaassie Blue to provide a reagent-filtrate admixture from which proteins may be determined; and,

said second reagent comprises methyl indole for determination of aldehydes in said eluant-reagent admixture.

39. The system of Claim 19 wherein said first analyte is polyphenols and said second analyte is lipid peroxides, and wherein:

said first membrane comprises microporous membrane which will prevent a portion of said matrix from passing therethrough, while allowing proteins and aldehydes to pass therethrough;

said second membrane is a membrane which captures lipid peroxides while allowing polyphenols to pass therethrough;

said first reagent is 2,2-diphenyl-1-picryl hydrazine to provide a reagent-filtrate admixture from which polyphenols may be determined; and,

said second reagent comprises xylenol orange with acidified iron for determination of lipid peroxides in said eluant-reagent admixture.

40. The system of Claim 19 wherein said first analyte is polyphenols and said second analyte is free fatty acids, and wherein:

said first membrane comprises microporous membrane which will prevent a portion of said matrix from passing therethrough, while allowing proteins and aldehydes to pass therethrough;

said second membrane is a membrane which captures free fatty acids while allowing polyphenols to pass therethrough;

said first reagent being selected from the group of reagents consisting of:

folin ciocalteau; and,

NH<sub>3</sub> with Fe<sup>++</sup>

12 for determination of polyphenols in said eluant-reagent admixture; and,  
13 said second reagent being xylenol orange to provide a reagent-filtrate  
14 admixture from which free fatty acids may be determined.

1 41. The system of Claim 19 wherein said first analyte is lipid peroxides  
2 and said second analyte is polyphenols, and wherein:

3 said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 proteins and aldehydes to pass therethrough;

6 said second membrane is a membrane which captures polyphenols  
7 while allowing lipid peroxides to pass therethrough;

8 said first reagent is xylenol orange with acidified iron to provide a  
9 reagent-filtrate admixture from which lipid peroxides may be determined;  
10 and,

11 said second reagent comprises Prussian Blue in  $H_3PO_4$  with EDTA for  
12 determination of polyphenols in said eluant-reagent admixture.

1 42. The system of Claim 18 wherein said analyte is procymidone, and  
2 wherein:

3 said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 procymidone to pass therethrough; and,

6 said system further comprises a second membrane positioned after  
7 said first membrane, said second membrane being a membrane which  
8 removes pigments while allowing procymidone to pass therethrough; and,

9 said reagent is  $H_2O_2$  and tetramethyl benzidine to provide a filtrate-  
10 reagent admixture from which procymidone may be determined.

1 43. The system of Claim 22 wherein said sub-detectable analyte is metals,  
2 and wherein:

3 said first membrane comprises microporous membrane which will  
4 prevent a portion of said matrix from passing therethrough, while allowing  
5 metals to pass therethrough; and,  
6 said concentrating membrane is a membrane which captures metals; and  
1 metals captured on the membrane are subsequently released from  
2 said membrane by an  $\text{Fe}^{+3}$  solution; and,  
3 said reagent is xylenol orange to provide a flush solution-reagent  
4 admixture from which metals may be determined.

1 44. A method for determining histamine in a sample, said method  
2 comprising the steps of:

3 A. adding to the sample a reagent which causes histamine to oxidize with  
4 resultant production of  $\text{H}_2\text{O}_2$ ; and, thereafter,

5 B. determining  $\text{H}_2\text{O}_2$  in the sample as an indicator of histamine which  
6 was present prior to oxidation.

1 45. The method of Claim 44 wherein the reagent used to oxidize the  
2 histamine in step A is diamine oxidase.

1 46. The method of Claim 44 wherein the  $\text{H}_2\text{O}_2$  is determined in step B by  
2 adding xylenol orange and acidified iron to the sample, and subsequently  
3 determining  $\text{H}_2\text{O}_2$  based on the change in color of the xylenol orange.

1 47. The method of Claim 46 wherein the change in color of the xylenol  
2 orange is determined by a determination method selected from the group  
3 consisting of:

4 visual determination; and,

5 spectral determination.

1 48. The method of Claim 44 wherein steps A and B are carried out by  
2 adding diamine oxidase + xylenol orange + acidified iron to the sample.



1 49. The method of Claim 48 wherein the formulation of the diamine  
2 oxidase + xylenol orange + acidified iron comprises:

3 diamine oxidase.....1000IU  
4 xylenol orange.....0.1% by weight  
5 acidified Fe<sup>++</sup> .....1-10 m mol.

1 50. The method of Claim 48 wherein the diamine oxidase + xylenol orange  
2 + acidified iron is solubilized in a mixture of buffered water and isopropanol.

1 51. A method for determining free fatty acids in a sample, said method  
2 comprising the steps of:

- 3 A. adding a quantity of xylenol orange to the sample; and,  
4 B. determining the change in color of the xylenol orange to indicate  
5 free fatty acids.

1 52. The method of Claim 51 wherein the xylenol orange is added to a  
2 concentration of between 0.1 % and 10.0 % by weight.

1 53. The method of Claim 51 wherein the xylenol orange is solubilized in  
2 water.

1 54. The method of Claim 51 wherein the xylenol orange is solubilized in  
2 isopropanol and water.

55. A method for determining free fatty acids in a sample, said method  
comprising the steps of:

- A. adding a quantity of thymol blue to the sample; and,  
B. determining the change in color of the thymol blue to indicate  
free fatty acids.

1           56. A method for determining lipid peroxides in a sample, said method  
2 comprising the steps of:

3           A. adding to the sample a quantity of hemoglobin and an activated  
4 electron donor substance, such that lipid peroxides present in the sample will cause  
5 at least some of the hemoglobin to convert to a modified hemoglobin derivative;  
6 and,

7           B. determining the amount of modified hemoglobin derivative present as  
8 an indication of lipid peroxides in the sample.

1           57. The method of Claim 56 wherein the activated electron donor  
2 substance in step A is acidified iron.

1           58. The method of Claim 56 wherein step B is carried out by visual  
2 determination of the change in color of the hemoglobin.

1           59. The method of Claim 56 wherein step B is carried out by spectral  
2 determination of the hemoglobin derivative.

1           60. The method of Claim 59 wherein said spectral determination is carried  
2 out at approximately 400 nanometers.

1           61. The method of Claim 56 wherein step A is carried out by adding to the  
2 sample a hemoglobin reagent having the formula:

3  
4                   hemoglobin.....0.01-5.0 % by weight

5                   iron.....2-20 m mol

1           62.    The method of Claim 56 wherein step A is carried out by adding to the  
2 sample a hemoglobin reagent which contains 0.01-10.0 % by weight hemoglobin  
3 and 2-20 % by weight iron, in a buffered solution.

1           63.    A method of determining sulfite and/or bisulfite in a sample, said  
2 method comprising the steps of:

3           A.     adding a trivalent iron-xylene orange complex to the sample; and,  
4           B.     determining the change in color of the trivalent iron-xylene orange  
5 complex as an indicator of sulfite and/or bisulfite in the sample.

1           64.    The method of Claim 63 wherein step B is carried out by a detection  
2 method selected from the group consisting of:

3                   visual determination; and,  
4                   spectral determination.

1           65.    The method of Claim 64 wherein the detection method is spectral and  
2 is carried out at 570 nanometers.

1           66.    The method of Claim 65 wherein step A is carried out by adding to the  
2 sample a reagent containing 0.1-5.0 % by weight of  $\text{Fe}^{+3}$  (xanthine oxidase) in  
3 water/isopropanol solution.

1           67.    The apparatus according to Claim 1 wherein at least some of the  
2 membrane modules are configured so as to nest within one another when stacked,  
3 thereby ensuring proper alignment of the membrane modules to allow sample to  
4 flow through each sample flow channel.

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A36

Add  
A37